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PATENT & TRADEMARK OFFICE

#23
Declaration
S. Boyce
10/7/03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Boyce et al.
EXAMINER: Prebilic, P.

SERIAL NO.: 09/543,268
GROUP ART UNIT: 3738

FILED: April 5, 2000
DOCKET: 285-79 CON

FOR: OSTEOIMPLANT AND METHOD FOR ITS MANUFACTURE

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TECHNOLOGY CENTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF TODD M. BOYCE
AND ALBERT MANRIQUE UNDER 37 C.F.R. § 1.131

Sir:

We, Todd M. Boyce, Ph.D., and Albert Manrique, declare and say as follows:

1. Todd M. Boyce is a Senior Scientist in the employ of Osteotech, Inc., the assignee of record of the subject patent application, and a named inventor therein.

2. Albert Manrique was, during the period of all of the acts hereinafter described, a Senior Research Scientist in the employ of Osteotech, Inc., and a named inventor in the subject patent application.

3. In the Office Action mailed April 2, 2003, the Examiner has maintained the rejection of Claims 1-7, 9-21, 23-43, 45-61, 63-80 and 82-134 of the subject patent application under 35 U.S.C. § 102(e) as unpatentable over Boyce et al. U.S. Patent No. 5,899,939 ("Boyce et al. '939") which issued on May 4, 1999 on original application Serial No. 09/009,997 filed January 21, 1998.

4. Applicants filed a Response to the Office Action on May 16, 2003 accompanied by the Combined Declaration of Todd M. Boyce and Albert Manrique under 37 C.F.R.§1.131. In the Advisory Action mailed May 28, 2003, the Examiner stated that "[t]he declaration was not sufficient to show that the applicant had completed the invention, that it worked for its intended purpose, and that it had the claimed properties such as compression strength."

5. The subject application was filed on April 5, 2000 as a continuation of U.S. patent application Serial No. 09/020,205 filed February 6, 1998, which issued as U.S. Patent No. 6,123,731 on September 26, 2000. The subject application is entitled to an effective filing date of February 6, 1998.

6. We make this declaration under 37 C.F.R. § 1.131 in order to present a showing of facts evidencing the making of the claimed invention in this country prior to the January 21, 1998 filing date of the application underlying the grant of the Boyce et al. '939 patent. Specifically, we make this declaration in order to present a showing of facts which, in character and weight, establish the conception of the invention of Claim 1 and that of the other rejected claims prior to the January 21, 1998 filing date of the aforesaid Boyce et al. application coupled with due diligence from prior to said date to the February 6, 1998 effective filing date of the subject application.

7. All of the acts hereinafter described took place in the United States.

8. Attached hereto is Exhibit A, a copy of (1) a facsimile draft patent application received from outside patent counsel, each page of which bears the date of January 9, 1998, as marked up by Dr. Boyce, (2) outside patent counsel's facsimile cover

sheet dated January 9, 1998 for the draft patent application as sent to Dr. Boyce and (3) an internal memorandum from Dr. Boyce dated January 9, 1998 attaching documents (1) and (2) for distribution to the individuals listed in the memorandum ("distribution list") and requesting their review and comments by January 19, 1998. The draft patent application as marked up by Dr. Boyce evidences the conception of the invention of amended Claim 1 herein prior to the January 21, 1998 filing date of the application underlying the grant of the Boyce et al. '939 patent as shown by the following side-by-side comparison of the elements of Claim 1 of the subject application and corresponding disclosure in the marked-up draft patent application constituting part of Exhibit A:

Claim 1 of the Subject Application	Marked-up Draft Patent Application
1. An osteoimplant which comprises a solid aggregate of bone-derived elements selected from the group consisting of superficially demineralized bone-derived elements, substantially completely demineralized bone-derived elements and mixtures thereof, adjacent bone-derived elements being bonded to each other through chemical linkages between their surface-exposed collagen, provided, that where substantially all of the bone-derived elements are substantially completely demineralized bone-derived elements the osteoimplant contains at least one additional component selected from the group consisting of reinforcing particles and fillers, and wherein the solid aggregate of bone-derived elements possesses a compression strength of from about 10 to about 200 MPa.	Page 5, lines 1-7 discloses: In keeping with these and other objects of the invention, there is provided an osteoimplant which comprises a solid aggregate of bone-derived elements initially presenting surface-exposed collagen, with adjacent bone-derived elements being bonded to each other through chemical linkages between their surface-exposed collagen. Page 6, lines 15-21 discloses: The expression "surface-exposed collagen" shall be understood to refer to the result obtained by demineralizing the aforementioned bone-derived elements, the demineralization ranging from substantially complete (in which case the bone-derived elements are primarily collagen) to superficial (in which case only the surfaces of the bone-derived elements present exposed collagen).

Claim 1 of the Subject Application	Marked-up Draft Patent Application
	Page 9, lines 15-18 discloses: In addition to containing bone-derived elements, the osteoimplant of this invention can optionally possess one or more other components such as reinforcing particles, fibers, fillers, bone-growth inducing substances... Page 9, lines 7-14 discloses: Accordingly, when an osteoimplant exhibiting relatively high compression strength is desired, e.g., on the order of from about 20 to about 200 MPa, and preferably from about 40 to about 150 MPa, it is necessary to employ bone-derived elements which retain a high proportion of their original mineral content or, stated another way, which have only been superficially demineralized.

A similar side-by-side comparison will show that the subject matter of the other claims presented herein is described by the marked-up draft application constituting part of Exhibit A.

9. From January 9, 1998, the date Dr. Boyce distributed Exhibit A to those on the distribution list referred to in paragraph 8, to on or about January 28, 1998, the marked-up draft was undergoing review by individuals on the distribution list. On January 28, 1998, Dr. Boyce sent a facsimile memorandum to outside counsel with a list

of proposed changes to be made to the draft application. Attached hereto as Exhibit B is a copy of this memorandum and its cover sheet.

10. Between January 28, 1998 and February 5, 1998, outside counsel revised the draft application based at least in part on the contents of Exhibit B and forwarded the revised application, with formal documents, to applicants for their review and prospective execution. On February 5, 1998, as evidenced by Exhibit C (cover letter referencing the executed application), applicants executed the application returning same to outside counsel with instructions to file in the PTO.

11. The application was filed on February 6, 1998 as Serial No. 09/020,205. This application, of which the subject application is a continuation, is a constructive reduction to practice of the invention disclosed in the marked-up draft application constituting part of Exhibit A.

12. The facts presented in paragraphs 8 to 11 and the referenced documentary exhibits establish conception of the invention of the claims herein prior to the January 21, 1998 filing date of the Boyce et al. '939 patent coupled with due diligence from prior to said date to the filing of application Serial No. 09/020,205 on February 6, 1998.

13. We each further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under

§ 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: Sept 26, 2003

Todd M. Boyce

Dated:__________, 2003

Albert Manrique

JCT:mg

Exhibit A

OSTEOTECH

Memorandum

CONFIDENTIAL

DATE: January 9, 1998

TO: Jack Boyle
Michael Dowd
Perry Geremikas
Jean Edwards
David Kaes
Albert Manrique
Richard Russo
Jim Russell
Nelson Scarborough
Rick Wright

FROM: Todd Boyce

RE: "Cross-linking" patent application

Attached is a first draft of our patent application for the cross-link adhesion methodology and implants made by cross-linking. Please review it. If you have suggestions for changes or if there are aspects which are not covered that we should consider, then please contact me with suggested alterations. I would like to collect all comments by Monday morning, January 19, so that I can get back to our patent attorney with revisions and prepare for submission. If you have any questions, I can be reached at ext. 6235. Thank you.

98_0109a

DILWORTH & BARRESE

ATTORNEYS AT LAW

333 EARLE OVINGTON BOULEVARD
UNIONDALE, NEW YORK 11553

TELEPHONE: (516) 228-8484
FACSIMILE: (516) 228-8516
e-mail: iplaw@dilworthbarrese.com

PETER G. DILWORTH
ROCCO S. BARRESE
DAVID M. CARTER
PAUL J. FARRELL
PETER DeLUCA
FRANK CHAU

ADRIAN T. CALDERONE
GEORGE M. KAPLAN
JEFFREY S. STEEN
JOSEPH W. SCHMIDT
RAYMOND E. FARRELL
RUSSELL R. KASSNER
CHRISTOPHER G. TRAINOR*
GLENN F. SAVIT
GEORGE LIKOUREZOS
JAMES M. LOEFFLER
WILLIAM E. LEWIS
JAMES J. BITETTO
FRANK J. MARTINEZ
JOHN G. TUTUNJIAN

*ADMITTED IN NEW JERSEY ONLY

OF COUNSEL
FERNANDA M. FIORDALISI

CALIFORNIA OFFICE

4350 LA JOLLA VILLAGE DR.
SUITE 300
SAN DIEGO, CA 92122
TELEPHONE: (619) 546-4410
FACSIMILE: (619) 558-9498
(619) 453-2839

OF COUNSEL
FREDERICK W. PEPPER

VIRGINIA OFFICE

TELEPHONE: (703) 978-6610
FACSIMILE: (703) 978-5412
ADDRESS: POST OFFICE BOX 7187
FAIRFAX STATION, VA 22039

OF COUNSEL
JAMES F. POWERS, JR.

FACSIMILE TRANSMISSION

CONFIDENTIAL

DATE: January 9, 1998

TO: Todd M. Boyce, ph.D.
Osteotech Inc.

SUBJECT: OSTEOIMPLANT AND METHOD FOR ITS MANUFACTURE
Our Docket: 285-79

FROM: Peter Dilworth, Esq./Anthony Bottino, Esq.
DILWORTH & BARRESE

NO. OF PAGES TO FOLLOW: 34

MESSAGE:

Dear Todd:

We enclose a draft application herein of 285-79.

Peter

IN CASE OF INCOMPLETE OR INADEQUATE TRANSMISSION, PLEASE CALL (516) 228-8484.

Confidentiality Notice: The information contained in this facsimile message is legally privileged and/or confidential information intended only for the use of the individual or entity named above. If you are not the intended recipient, you are hereby notified that any use, dissemination, distribution, or copying of this facsimile or its content is strictly prohibited. If you have received this facsimile in error, please immediately notify us by telephone and return the original facsimile message to us by mail or destroy it without making a copy. Thank you.

OSTEOIMPLANT AND METHOD FOR ITS MANUFACTURE

BACKGROUND OF THE INVENTION

Field of Invention

The present invention relates to an osteoimplant for use in the repair, replacement and/or augmentation of various portions of animal or human skeletal systems and to a method for manufacturing the osteoimplant. More particularly, this invention relates to an osteoimplant made up of a solid aggregate of bone-derived elements that are bonded to each other through chemical linkages formed between their surface-exposed collagen.

Description of the Related Art

The use of autograft bone, allograft bone or xenograft bone is well known in both human and veterinary medicine. See Stevenson et al., *Clinical Orthopedics and Related Research*, 323, pp. 66-74 (1996). In particular, transplanted bone is known to provide support, promote healing, fill bony cavities, separate bony elements such as vertebral bodies, promote fusion and stabilize the sites of fractures. More recently, processed bone has been developed into shapes for use in new surgical applications, or as new materials for implants that were historically made of non-biologically derived materials.

U.S. Patent No. 4,678,470 describes a non-layered bone grafting material produced from bone by a process which includes tanning with glutaraldehyde. The bone may be pulverized, used as a large block or machined into a precise shape. The tanning stabilizes the material and also renders it non-antigenic. The bone material may also be demineralized.

Collagen is a naturally occurring structural biomaterial and is a component of connective tissues, including bone, in all vertebrate species. Native collagen is a glycine-rich chain of amino acids arranged in a triple helix and can be cross-linked by a variety of procedures.

Tissue transglutaminase is described as being effective at increasing adhesive strength at a cartilage-cartilage interface. See Jurgensen, K., et al., *The Journal of Bone and Joint Surgery*, 79-A (2), 185-193 (1997).

U.S. Patent No. 5,507,813 describes a surgically implantable sheet formed from elongate bone particles, optionally demineralized, containing biocompatible ingredients, adhesives, fillers, plasticizers etc.

U.S. Patent No. 4,932,973 discloses an artificial organic bone matrix with holes or perforations extending into the organic bone matrix. The holes or perforations are indicated to be centers of cartilage and bone

Missing text?

demineralized bone powder or micro particulate bone, and reconstituted collagen. The sponge-like graft is optionally cross-linked with glutaraldehyde.

Another one-piece porous implant is described in U.S. Patent No. 5,683,459. The implant is made up of a biodegradable polymeric macrostructure, which is structured as an interconnecting open cell meshwork, and a biodegradable polymeric microstructure composed of chemotactic ground substances such as hyaluronic acid.

SUMMARY OF THE INVENTION

The present invention provides an osteoimplant which, due to chemical linkages formed between the surface-exposed collagen of adjacent partially demineralized bone elements from which the osteoimplant is manufactured, exhibits good mechanical strength, is biocompatible and, in a preferred embodiment, through its bone healing activity and ability to contain bone-growth inducing substances, can promote and/or accelerate new bone growth.

It is therefore an object of the present invention to provide an osteoimplant made up of a solid aggregate of adjacent bone-derived elements bonded to each other

through chemical linkages between their initially presented surface-exposed collagen, and which possesses good mechanical strength and biocompatibility.

It is another object of this invention to provide an osteoimplant which can optionally include another component such as a reinforcing particle or fiber, fillers, bone-growth inducing substances such as medically/surgically useful substances, and combinations thereof.

It is another object of the invention to provide an osteoimplant possessing a network of pores, perforations, apertures, channels or spaces which permits and encourages penetration by endogenous and exogenous bone healing materials and blood supply, and simultaneously provides a means for incorporating one or more bone healing substances.

It is yet a further object of the present invention to provide an osteoimplant which can be fashioned into a variety of shapes and sizes which are not limited by constraints imposed by the size and/or types of donor bone which are available for construction of the osteoimplant.

It is also an object of the invention to provide a method of manufacturing which will provide a strong, biocompatible osteoimplant of any size and/or shape for implantation.

In keeping with these and other objects of the invention, there is provided an osteoimplant which comprises a solid aggregate of bone-derived elements initially presenting surface-exposed collagen, with adjacent bone-derived elements being bonded to each other through chemical linkages between their surface-exposed collagen.

Further in keeping with the invention, there is provided a method for the manufacture of an osteoimplant which comprises providing a quantity of bone-derived elements presenting surface-exposed collagen and forming chemical linkages between the surface-exposed collagen to bond the elements into a solid aggregate.

The osteoimplant of the present invention possesses a significant advantage over the prior art in its ability to be biocompatible, non-antigenic and good to provide mechanical strength.

Another important advantage of the osteoimplant herein over prior art implants lies in its ability to function as a carrier for, and effectively diffuse, one or more bone-growth inducing substances that promote new bone growth and/or accelerate healing.

The term "osteogenic" as used herein shall be understood to refer to the ability of a substance to induce new bone formation via the participation of living cells from within the substance.

The term "osteoinductive" as used herein shall be understood to refer to the ability of a substance or material to provide biologically inert surfaces which are receptive to the growth of new host bone.

The term "osteoconductive" as used herein shall be understood to refer to the ability of a substance to recruit cells from the host which have the potential for repairing bone tissue.

Use of the expression "bone-derived elements" shall be understood to refer to pieces of bone in any variety of sizes, thicknesses and configurations including particles, strips, thin to thick sheets, etc., which can be obtained by milling, slicing, cutting or machining whole bone.

The expression "surface-exposed collagen" shall be understood to refer to the result obtained by demineralizing the aforementioned bone-derived elements, the demineralization ranging from substantially complete (in which case the bone-derived elements are primarily collagen) to superficial (in which case only the surfaces of the bone-derived elements present exposed collagen).

BRIEF DESCRIPTION OF THE DRAWINGS

Various embodiments are described below with reference to the drawings wherein:

FIG. 1 is a cross-sectional view of bone from the diaphyseal region which has been sliced longitudinally into several cortical bone sheets;

FIG. 2 is an enlarged perspective view of an osteoimplant of the invention possessing sheets of partially demineralized bone at their surface and an interior made up of mineralized or partially demineralized bone;

FIG. 3 is a view of a human femur showing an osteoimplant of the invention, as shown in FIG. 3A, fashioned as a femural bone replacement;

FIG. 4 is a partial view of the human vertebral column showing a disc-shaped osteoimplant of the invention installed at an intervertebral site;

FIGS. 5 and 5A are views of a human skull showing an osteoimplant of the invention fashioned as a parietal bone replacement;

FIG. 6 is an enlarged perspective view of an osteoimplant of the invention possessing alternating layers of bone sheets and cubes with channels between the cubes.

FIG. 7 is a partial view of the human vertebral column showing installation of the osteoimplant of Fig.6

at a posterolateral intertransverse process fusion site; and,

FIG. 8A is an enlarged perspective view of an osteoimplant of the invention possessing layers of bone sheets bonded together via chemical bonds formed by catalysis with tissue transglutaminase, as shown in FIG. 8.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The osteoimplant of the present invention comprises a solid aggregate of bone-derived elements having chemical linkages between their initially surface-exposed collagen molecules thus bonding adjacent bone elements to each other. In order to expose the collagen located on the outer surface of bone, the bone elements must be at least partially demineralized. Demineralization methods remove the mineral component of bone employing acid solutions. Such methods as used by the present invention are well known in the art, see for example, Reddi et al., *Proc. Nat. Acad. Sci.* 69, pp1601-1605 (1972), incorporated herein by reference. The strength of the acid solution, the shape of the bone and the duration of the demineralization treatment will determine the extent of demineralization. Reference in this regard may be made to Lewandrowski et al., *J. Biomed Materials Res*, 31, pp365-372 (1996), also incorporated herein by reference. The sources for the bone-derived elements herein include

cortical and cancellous bone and are preferably allogenic but also include xenogenic sources such as bovine and porcine bone.

When prepared from bone-derived elements that are only superficially demineralized, the osteoimplant herein will tend to possess a fairly high compression strength, e.g., one approaching that of natural bone. Accordingly, when an osteoimplant exhibiting relatively high compression strength is desired, e.g., on the order of from about 20 to about 200 MPa, and preferably from about 40 to about 150 MPa, it is necessary to employ bone-derived elements which retain a high proportion of their original mineral content of, or stated another way, which have only been superficially demineralized.

In addition to containing bone-derived elements, the osteoimplant of this invention can optionally possess one or more other components such as reinforcing particles, fibers, fillers, bone-growth inducing substances, adhesives, plasticizers, flexibilizing agents, hydration facilitating agents, biostatic/biocidal agents, substances imparting radiopacity, metallic meshes and the like. Examples of reinforcing particles include cortical and cancellous bone, and partially or fully demineralized cortical and cancellous bone. Examples of fillers include mineral material such as hydroxyapatite, tricalcium phosphate and other calcium salts, bone

powder, demineralized bone powder, bioglass or other bioceramic or natural or synthetic polymers, e.g., bioabsorbable polymers such as polyglycolide, polylactide, glycolide-lactide copolymer, and the like, and nonbioabsorbable polymers such as **[PLEASE FILL IN]**. From 77 applica Suitable plasticizers, flexibilizing agents and hydration facilitating agents, include liquid polyhydroxy compounds such as glycerol, monacetin, diacetin, and mixtures thereof. Suitable biostatic/biocidal agents include antibiotics, povidone, sugars, and mixtures thereof; suitable surface agents include the biocompatible nonionic, cationic, anionic and amphoteric surfactants, and mixtures thereof. The osteoimplant can also possess bone-growth inducing substances which include any of a variety of medically and/or surgically useful substances which are described below.

The osteoimplant can possess one or more cavities which, if desired, can communicate with the surface of the implant through pores, apertures, perforations or channels provided for this purpose and ranging in average diameter from a few microns to several millimeters. Such cavities and their associated pores, apertures, perforations, and channels can be partially or completely filled with one or more medically/surgically useful substances which promote or accelerate new bone growth or bone healing due, e.g., to some osteogenic,

osteoconductive and/or osteoconductive effect. Useful substances of this kind which can be incorporated into the osteoimplant of this invention include, e.g., collagen, insoluble collagen derivatives, etc., and soluble solids and/or liquids dissolved therein, e.g., antiviral agents, particularly those effective against HIV and hepatitis; antimicrobials and/or antibiotics such as erythromycin, bacitracin, neomycin, penicillin, polymyxin B, tetracyclines, viomycin, chloromycetin and streptomycins, cefazolin, ampicillin, azactam, tobramycin, clindamycin and gentamicin, etc.; biocidal/biostatic sugars such as dextroal, glucose, etc.; amino acids, peptides, vitamins, inorganic elements, co-factors for protein synthesis; hormones; endocrine tissue or tissue fragments; synthesizers; enzymes such as collagenase, peptidases, oxidases, etc.; polymer cell scaffolds with parenchymal cells; angiogenic drugs and polymeric carriers containing such drugs; collagen lattices; antigenic agents; cytoskeletal agents; cartilage fragments, living cells such as chondrocytes, other cells bone marrow cells, mesenchymal stem cells, natural extracts, tissue transplants, bone, demineralized bone powder (or "demineralized bone matrix" as it may also be referred to), autogenous tissues such as blood, serum, soft tissue, bone marrow, etc.; bioadhesives, bone morphogenic proteins (BMPs), transforming growth factor

Angiogenic

(TGF-beta), insulin-like growth factor (IGF-1); growth hormones such as somatotropin; bone digestors; antitumor agents; immuno-suppressants; permeation enhancers, e.g., fatty acid esters such as laureate, myristate and stearate monoesters of polyethylene glycol, enamine derivatives, alpha-keto aldehydes, etc.; and, nucleic acids. These and similar medically/surgically useful substances can be incorporated into the osteoimplant of this invention or any of its constituent bone-derived elements or other components during any stage of the assembly of the implant. Suitable methods of incorporation include coating, immersion saturation, packing, etc. The amounts of medically/surgically useful substances utilized can vary widely with optimum levels being readily determined in a specific case by routine experimentation.

Osteoimplants of any desirable size and/or configuration can be provided, e.g., by machining or other mechanical shaping operations such as press-molding. Computerized modeling of a specific implant followed by computerized control of the shaping of the implant can be used to provide an intricately shaped osteoimplant which is custom-fitted to the intended site of application with great precision.

Where the invention comprises aggregates of elongate bone-derived elements which, in appearance can be

described as filaments, fibers, threads, slender or narrow strips, etc., an osteoimplant can be formed from these elements by a variety of methods. For example, forming a solution or slurry in a suitable medium which can comprise the cross-linking agent, and any proportion of the elongate bone-derived elements being partially or fully demineralized, and fully mineralized. This solution can be formed into an osteoimplant of any shape according to the configuration of a mold into which it is poured. The mold is preferably shaped as a bone or section thereof or an implant shape. Once contained in a mold, the solution of bone-derived elements can be solidified into a solid osteoimplant by known techniques.

It is within the scope of the invention to supplement or increase the shape-retaining and/or mechanical strength characteristics of the osteoimplant, e.g., by the addition of mechanical fasteners such as pins, screws, dowels, etc., which can be fabricated from natural or synthetic materials and bioabsorbable as well as nonbioabsorbale materials, by the use of laser tissue welding or ultrasonic bonding, and so forth. In those embodiments of the osteoimplant which are assembled from relatively large bone-derived elements such as sheets, such elements can be provided with mechanically interengaging features, e.g., tongue-and-groove or mortise-and-tenon features, which facilitate their

assembly into the final product and/or to fix the elements to each other in a more secured fashion.

The osteoimplant herein is intended to be applied at a bone defect site, e.g., one resulting from injury, defect brought about during the course of surgery, infection, malignancy or developmental malformation. The osteoimplant, suitably sized and shaped as required, can be utilized as a graft or replacement in a wide variety of orthopaedic, neurosurgical and oral and maxillofacial surgical procedures such as the repair of simple and compound fractures and non-unions, external and internal fixations, joint reconstructions such as arthrodesis, general arthroplasty, cup arthroplasty of the hip, femoral and humeral head replacement, femoral head surface replacement and total joint replacement, repairs of the vertebral column including spinal fusion and internal fixation, tumor surgery, e.g., deficit filling, discectomy, laminectomy, excision of spinal cord tumors, anterior cervical and thoracic operations, repair of spinal injuries, scoliosis, lordosis and kyphosis treatments, intermaxillary fixation of fractures, mentoplasty, temporomandibular joint replacement, alveolar ridge augmentation and reconstruction, inlay onlay? bone grafts, implant placement and revision, sinus lifts, etc. Specific bones which can be repaired or replaced with the osteoimplant herein include the ethmoid,

frontal, nasal, occipital, parietal, temporal, mandible, maxilla, zygomatic, cervical vertebra, thoracic vertebra, lumbar vertebra, sacrum, rib, sternum, clavicle, scapula, humerus, radius, ulna, carpal bones, metacarpal bones, phalanges, ilium, ischium, pubis, femur, tibia, fibula, patella, calcaneus, tarsal, and metatarsal bones.

Implantation in human or veterinary medical Applications (Earlier)

The method of manufacturing the osteoimplant of the present invention comprises providing a quantity of bone-derived elements presenting surface-exposed collagen and subsequently forming chemical linkages between the surface-exposed collagen of adjacent bone-derived elements to bond the elements into a solid aggregate. These chemical linkages can be formed employing a variety of known methods including chemical reaction, e.g., ~~dye-mediated photo-oxidation~~; the application of energy such as radiant energy, which includes irradiation by UV light or microwave energy, drying and/or heating; dehydrothermal treatment in which water is slowly removed while the bone tissue is subjected to a vacuum; and, enzymatic treatment to form. ~~which is the preferred method of forming~~ chemical linkages at any collagen-collagen interface.

to Later on

Heat? in dehydro

Chemical cross-linking agents include those that contain bifunctional or multifunctional reactive groups, and which react with functional groups on amino acids such as epsilon-amine functional group of lysine or

hydroxy-lysine, or the carboxyl functional groups of aspartic and glutamic acids. By reacting with multiple functional groups on the same or different collagen molecules, the reacting chemical cross-linking agent forms a reinforcing cross-bridge.

Suitable chemical cross-linking agents include: mono- and dialdehydes, including glutaraldehyde and formaldehyde; polyepoxy compounds such as glycerol polyglycidal ethers, polyethylene glycol diglycidal ethers and other polyepoxy and diepoxy glycidal ethers; tanning agents including polyvalent metallic oxides such as titanium dioxide, chromium dioxide, aluminum dioxide, zirconium salt, as well as organic tannins and other phenolic oxides derived from plants; chemicals for esterification of carboxyl groups followed by reaction with hydrazide to form activated acyl azide functionalities in the collagen; dicylcohexyl carbodiimide and its derivatives as well as other heterobifunctional cross-linking agents; hexamethylene diisocyanate; sugars, including glucose, will also cross-link collagen.

Glutaraldehyde cross-linked biomaterials have a tendency to over-calcify in the body. In this situation, should it be deemed necessary, calcification-controlling agents can be used with aldehyde cross-linking agents. These calcification-controlling agents include: dimethyl sulfoxide (DMSO), surfactants, diphosphonates, aminooleic

acid, and metallic ions, for example iron and aluminum. The concentrations of these calcification-controlling agents can be determined by routine experimentation by those skilled in the art.

Chemical cross-linking involves exposing the bone-derived elements presenting surface-exposed collagen to the chemical agent, either by placing the elements in a solution of the chemical agent, or by exposing them to the vapors of the chemical agent under conditions appropriate for the particular type of cross-linking reaction. Such conditions include: an appropriate pH and temperature, and for times ranging from minutes to days, depending upon the level of cross-linking desired, and the activity of the chemical agent. The chemical agent is then washed to remove all leachable traces of the chemical.

When enzymatic treatment is employed, useful enzymes include those known in the art which are capable of catalyzing cross-linking reactions on proteins or peptides, preferably collagen molecules, e.g., transglutaminase as described in Jurgensen et al., *The Journal of Bone and Joint Surgery*, 79-A (2), 185-193 (1997), herein incorporated by reference.

Formation of chemical linkages can also be accomplished by the application of energy [PLEASE PROVIDE DETAILS OF GENERAL STEP(s) AND ESSENTIAL CONDITIONS].

Another method for the formation of chemical linkages ~~is by~~ dehydrothermal treatment. [PLEASE PROVIDE DETAILS OF GENERAL STEP(s) AND ESSENTIAL CONDITIONS].

Referring to the drawings, as shown in FIG. 1, the cortical portion of bone 10 taken from the diaphyseal region is cut into cortical bone sheets 11 of varying width by slicing the bone longitudinally. If desired, cortical bone sheets 11 can be further cut to uniform size and shape, as in bone-derived sheets 21 of the osteoimplant 20 shown in FIG. 2.

What about particulate/Fiber forms?

FIG. 2 illustrates an osteoimplant 20 comprising cortical bone-derived sheets 21 having a fully or partially demineralized outer surface with surface-exposed collagen, and a nondemineralized or partially demineralized core 22. Alternatively, one or more bone-derived sheets can be made from substantially completely demineralized bone. Also, another component such as demineralized bone powder can be coated on the bone-derived sheets. The entire structure has cross-linked collagen on adjacent bone-derived sheets to provide increased adhesion between them. The total thickness of the osteoimplant will ordinarily be at least about ______ mm. Osteoimplant 20 can be cut, machined, and/or otherwise formed into any other desired shape or dimension for implantation into a body. Thus, as shown in FIG. 3A, a substantially cylindrically shaped

osteoimplant 30 can be made for use as a long bone segment replacement 31 for a femur 32 of FIG. 3. To form a cylinder, a substantially square or rectangular osteoimplant can be shaped on a lathe to the required diameter. A cavity can be formed by removing bone material with, for example, a drill, or, alternatively, a cavity can be formed by assembling appropriately configured layers of bone-derived elements.

As shown in FIG. 4, the disc-shaped osteoimplant 40 is shown inserted at the intervertebral fibrocartilage site 41 on the anterior side of vertebral column 42.

In FIG. 5, parietal osteoimplant 50 is sized and shaped to form part of the parietal bone for skull 51 in FIG. 5A.

In FIG. 6, osteoimplant 60 is built up from bone-derived sheet sections 61 of surface demineralized cortical bone, and from bone-derived cube sections 62 of surface demineralized cancellous bone of uniform, square cross section. These sheet and cube constituents are arranged in alternating layers as shown. After assembly, the structure is subjected to treatment for cross-linking. Because of the open structure of osteoimplant 60 resulting from the pattern of channels 63, the osteoimplant permits vascular penetration or host bone ingrowth therein and/or diffusion of one or more medically/surgical useful substances therefrom.

Osteoimplant 60 is shown installed as a spinal onlay graft attached via insertion of the transverse processes 71 into channels 63, for posterolateral intertransverse process fusion on vertebral column 70 of FIG. 7.

In FIG. 8A, osteoimplant 80 comprises bone-derived sheets 81 having a fully or partially demineralized outer surface. As shown in FIG. 8, a bone-derived sheet has one side coated with tissue transglutaminase 83 and, the mating surface of the adjacent sheet is coated with $CACl_2$ 82 solution. As osteoimplant 80 is assembled, contact between the two complimentary sides of bone-derived sheets results in tissue transglutaminase 83 catalyzing collagen cross-linking at the interface of adjacent bone-derived sheets 81.

The following examples are further illustrations of the osteoimplant of this invention.

Example 1

A cortical section of bone from the diaphyseal region was cut in the longitudinal direction while continuously wetted with water into approximately 1.5 mm thick sheets using a diamond-bladed saw. The cortical bone-derived sheets were then frozen to -70C and freeze-dried for 48 hours, and subsequently, were placed into excess 0.6 NH_4Cl solution for 1.5 hours with constant stirring, washed in water for 5 minutes, and soaked for

1.5 hours in BupH phosphate buffered saline. The bone-derived sheets were assembled into a layered structure held in a clamp. The clamped structure was then placed into a solution of 10% neutral, buffered formalin for 48 hours to cross-link the exposed collagen surfaces. After crosslinking, the clamp was removed, and the structure was placed in a water bath to rinse running water for several hours. The osteoimplant was cut to shape on a band saw, and then placed in an excess aqueous solution of glycerol. After seven hours, the excess glycerol solution was removed, and the osteoimplant was freeze-dried.

Example 2

Elongate bone-derived fibers were milled from cortical bone, and were fully demineralized in excess 0.6N HCl solution. These fibers were washed with water, and soaked in an aqueous solution of glycerol. Additionally, fully mineralized bone-derived fibers were added to the solution which was stirred and left for 12 hours at room temperature. The solution containing the soaked mineralized and demineralized bone-derived fibers were poured through a micron sieve to recover the fibers, which were then pressure-treated to 10,000-50,000 psi in a press for 15 minutes, and were then heated for 2 to 12 hours at 37-55 degrees C. The resulting osteoimplant

pellet was freeze-dried, and placed in polyethylene glycol diglycidal ether for 12 hours at room temperature.

Example 3

Bone-derived sheets derived from human cortical bone, approximately 1 mm thick by 7 mm wide by 50 mm long, were treated for 10 minutes in 0.6 N HCl to expose surface collagen. Bone-derived cubes derived from human cancellous bone, 10 mm x 10 mm, were treated to expose surface collagen at the outer borders of the cubes. All bone-derived sheets and cubes were washed in water. The pieces were assembled together with bone-derived sheets bordering the cubes, and clamped into place. The construct was then placed into a solution of 10% neutral buffered formalin for 3 hours to cross-link the surface-exposed collagen. The resulting osteoimplant was then washed in water, and cut to size on a band saw. See Fig. 6.

Example 4

Human cortical bone-derived sheets approximately 1 mm thick sheets were surface demineralized for 15 minutes in 0.6N HCl, then washed in running water. Tissue transglu-tarminase was reconstituted to give a 1 mg/ml solution. For each demineralized bone-derived sheet in the construct, the surface was blotted dry, then 40 $\mu l/cm^2$

area of the tissue transglutaminase was applied to one side and an equivalent volume of 0.1M $CaCl_2$ solution was applied to the mating surface of the next demineralized bone-derived sheet. This was repeated sequentially. The resulting osteoimplant was clamped and placed into a humidity chamber to promote cross-linking for approximately 30 minutes, then washed in water.

Example 5

Cortical bone-derived sheets, approximately 2 mm thick, were surface demineralized in 0.6N HCl solution for 1 hour with constant stirring. The bone-derived sheets were then coated with dry, demineralized bone powder having a particle size of 300 microns or less, and assembled into layers. The construct was clamped into place, and placed into a solution of 10% neutral buffered formalin for 12 hours to permit collagen cross-linking. The resulting osteoimplant was washed in water to remove excess chemicals.

IN THE CLAIMS

1. An osteoimplant which comprises a solid aggregate of bone-derived elements initially presenting surface-exposed collagen, adjacent bone-derived elements being bonded to each other through chemical linkages between their surface-exposed collagen.

2. The osteoimplant of Claim 1 wherein the bone-derived elements are superficially demineralized particles, strips or sheets of allogenic and/or xenogenic cortical bone.

3. The osteoimplant of Claim 1 wherein the bone-derived elements are substantially completely demineralized particles, strips or sheets of allogenic and/or xenogenic cortical bone.

4. The osteoimplant of Claim 1 containing at least one other component.

5. The osteoimplant of Claim 4 wherein the component is selected from the group consisting of reinforcing particle or fiber, filler, bone-growth inducing substance,

__________________ and __________________.

6. The osteoimplant of Claim 1 possessing a cross section for at least a portion of its length which is, or approximates, a circle, oval or polygon, the implant optionally possessing a cavity for at least a portion of its length.

7. The osteoimplant of Claim 1 configured as a graft.

8. The osteoimplant of Claim 1 configured as a replacement for a bone or section thereof.

9. The osteoimplant of Claim 8 configured as an intervertebral insert, a long bone, a cranial bone, a bone of the pelvis, or a bone of the hand or foot or section thereof.

10. The osteoimplant of Claim 1 wherein the chemical linkages are formed by chemical crosslinking, application of energy, dehydrothermal treatment or enzymatic treatment.

11. The osteoimplant of Claim 1 possessing a compression strength of from about ______ to about ______ [units].

12. The osteoimplant of Claim 1 possessing a compression strength of from about ______ to about ______ [units].

13. The osteoimplant of Claim 1 possessing a hydration-facilitating agent.

14. The osteoimplant of Claim 1 wherein the hydration-facilitating agent is glycerol.

15. A method for the manufacture of an osteoimplant which comprises:

a) providing a quantity of bone-derived elements initially presenting surface-exposed collagen; and,

b) forming chemical linkages between the surface-exposed collagen of adjacent bone-derived elements to bond said elements into a solid aggregate.

16. The method for the manufacture of an osteoimplant of Claim 15 wherein the bone-derived elements are substantially completely demineralized particles, strips or sheets of allogenic and/or xenogenic cortical bone.

17. The method of Claim 15 wherein the chemical linkages are formed by chemical crosslinking, application of energy, dehydrothermal treatment or enzymatic treatment.

18. The method of Claim 15 carried out in a mold.

19. The method of Claim 18 wherein the shaping surfaces of the mold are such as to provide an osteoimplant configured as a bone or section thereof.

20. The method of Claim 18 wherein the shaping surfaces of the mold are such as to provide an osteoimplant configured as an intervertebral insert, a long bone, a cranial bone a bone of the pelvis, or a bone of the hand or foot or section thereof.

21. The method of Claim 17 wherein the chemical linkages are formed by [recite general step(s)/essential conditions for chemical crosslinking].

22. The method of Claim 21 wherein the chemical-crosslinking agent is selected from the group consisting of ____________________ and ____________________.

23. The method of Claim 17 wherein the chemical linkages are formed [recite general step(s)/essential conditions for crosslinking by application of energy].

24. The method of Claim 17 wherein the chemical linkages are formed by [recite general step(s)/essential conditions for crosslinking by dehydrothermal treatment]..

25. The method of Claim 17 wherein the chemical linkages are formed by [recite general step(s)/essential conditions for crosslinking by enzymatic treatments].

ABSTRACT

The invention relates to an osteoimplant fabricated from a solid aggregate of bone derived elements possessing chemical linkages between their adjacent surface-exposed collagen. Also described are various other components which can be incorporated into the bone implant material such as bone-growth inducing substances; and a method of manufacture.

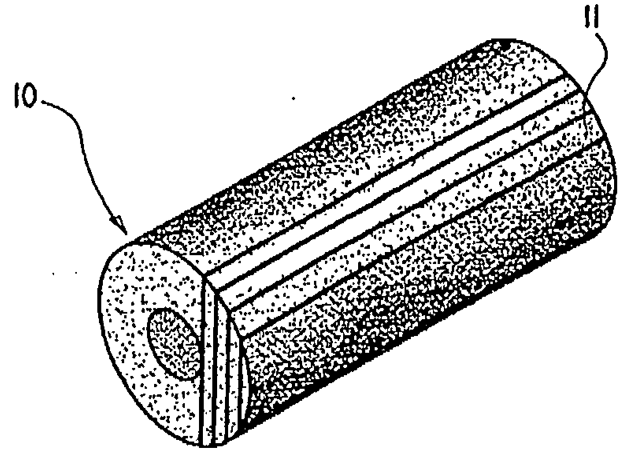

FIG. 1

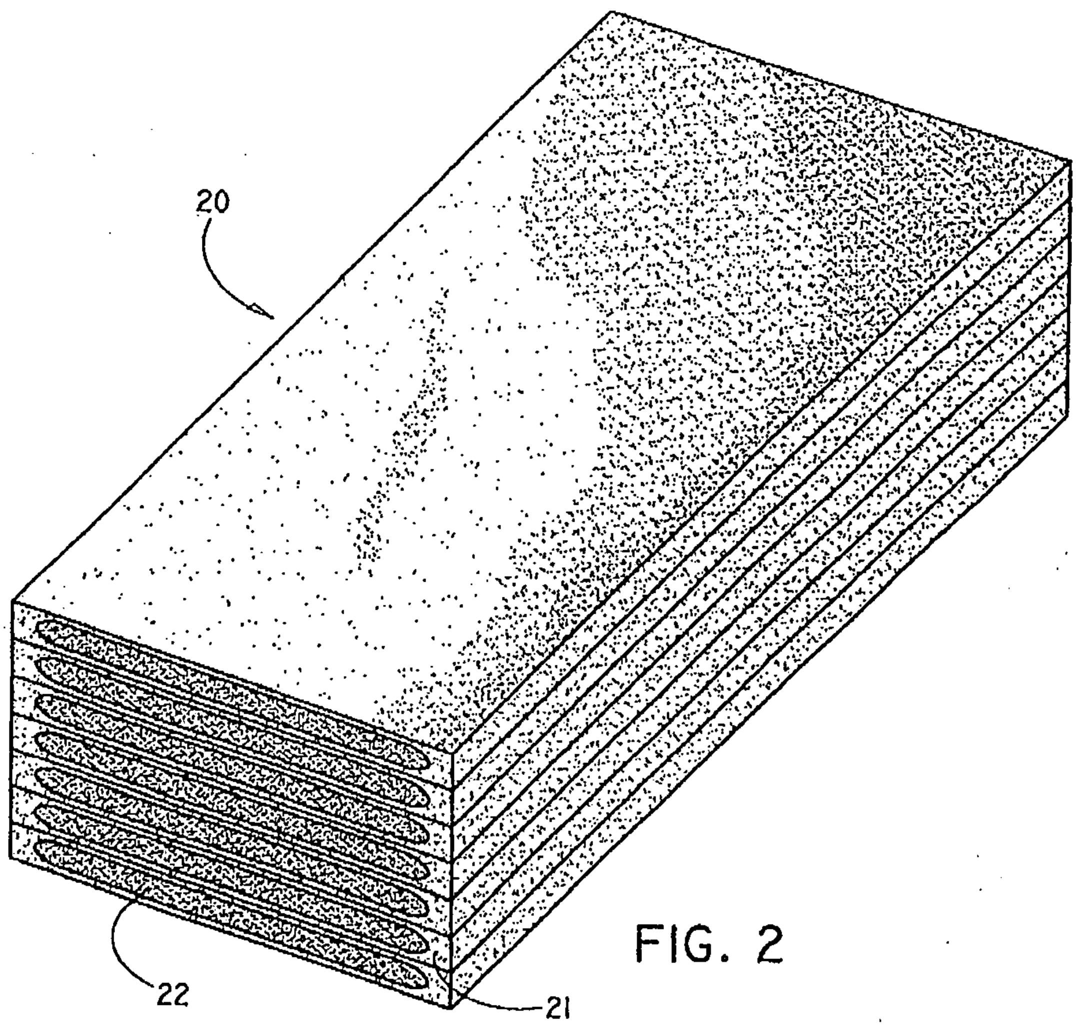

FIG. 2

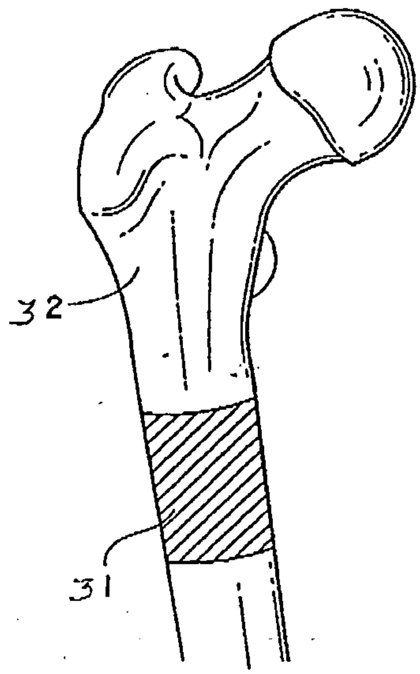

FIG. 3

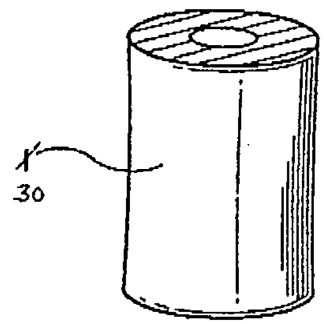

FIG. 3A

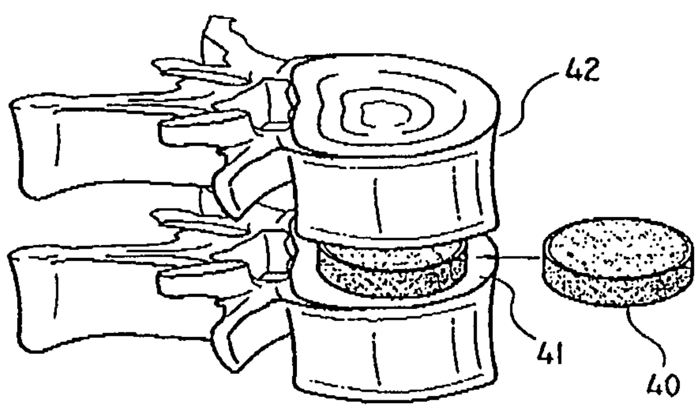

FIG. 4

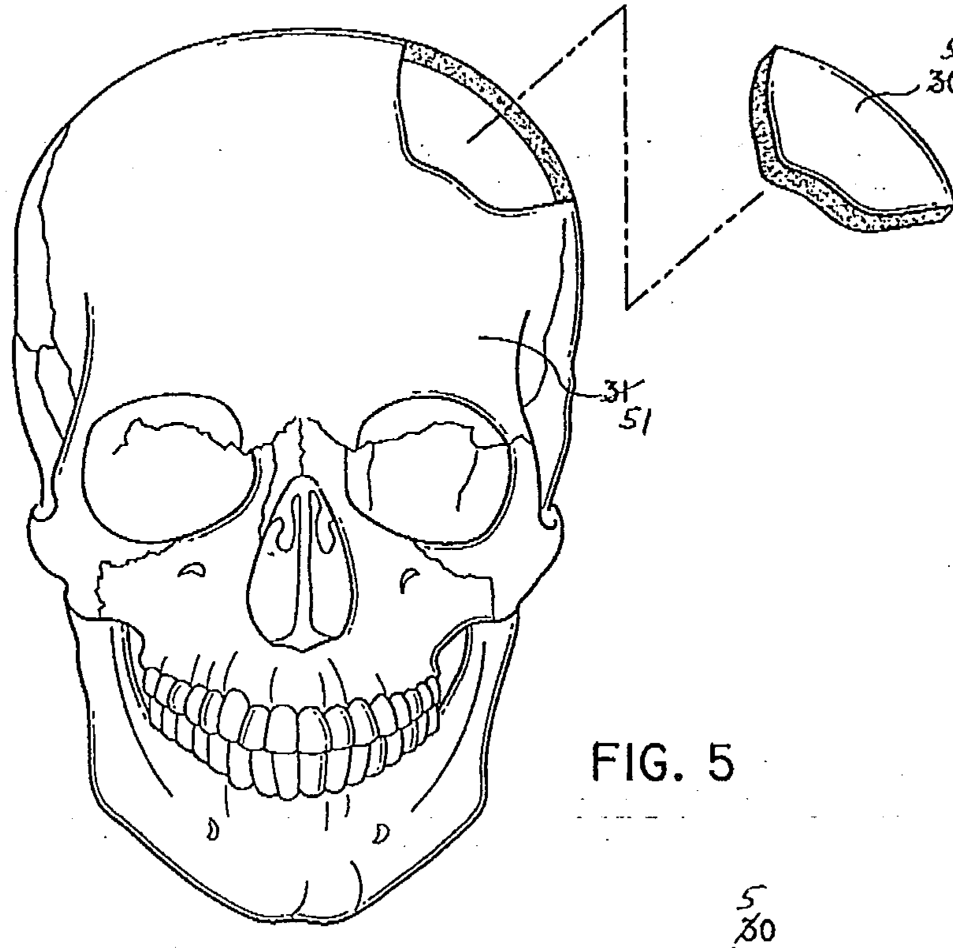

FIG. 5

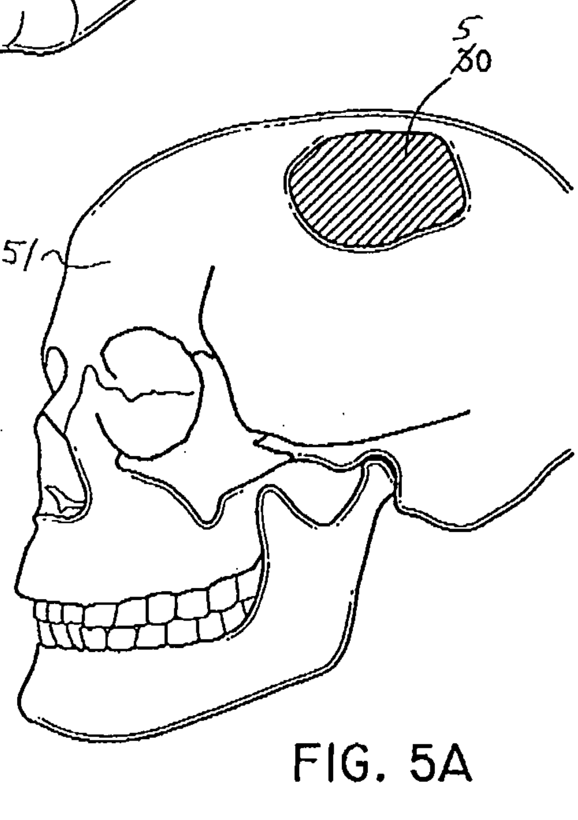

FIG. 5A

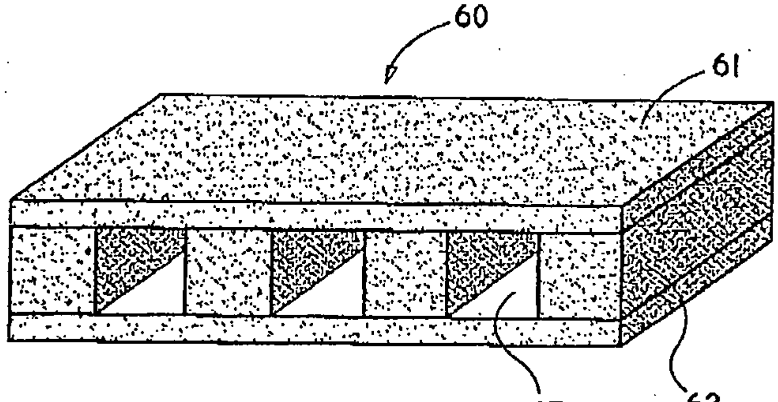

FIG. 6

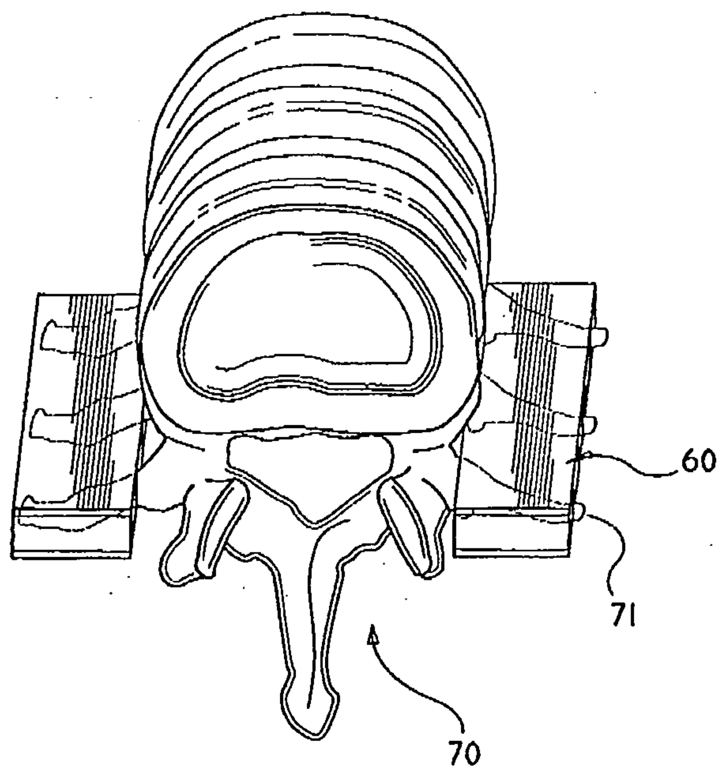

FIG. 7

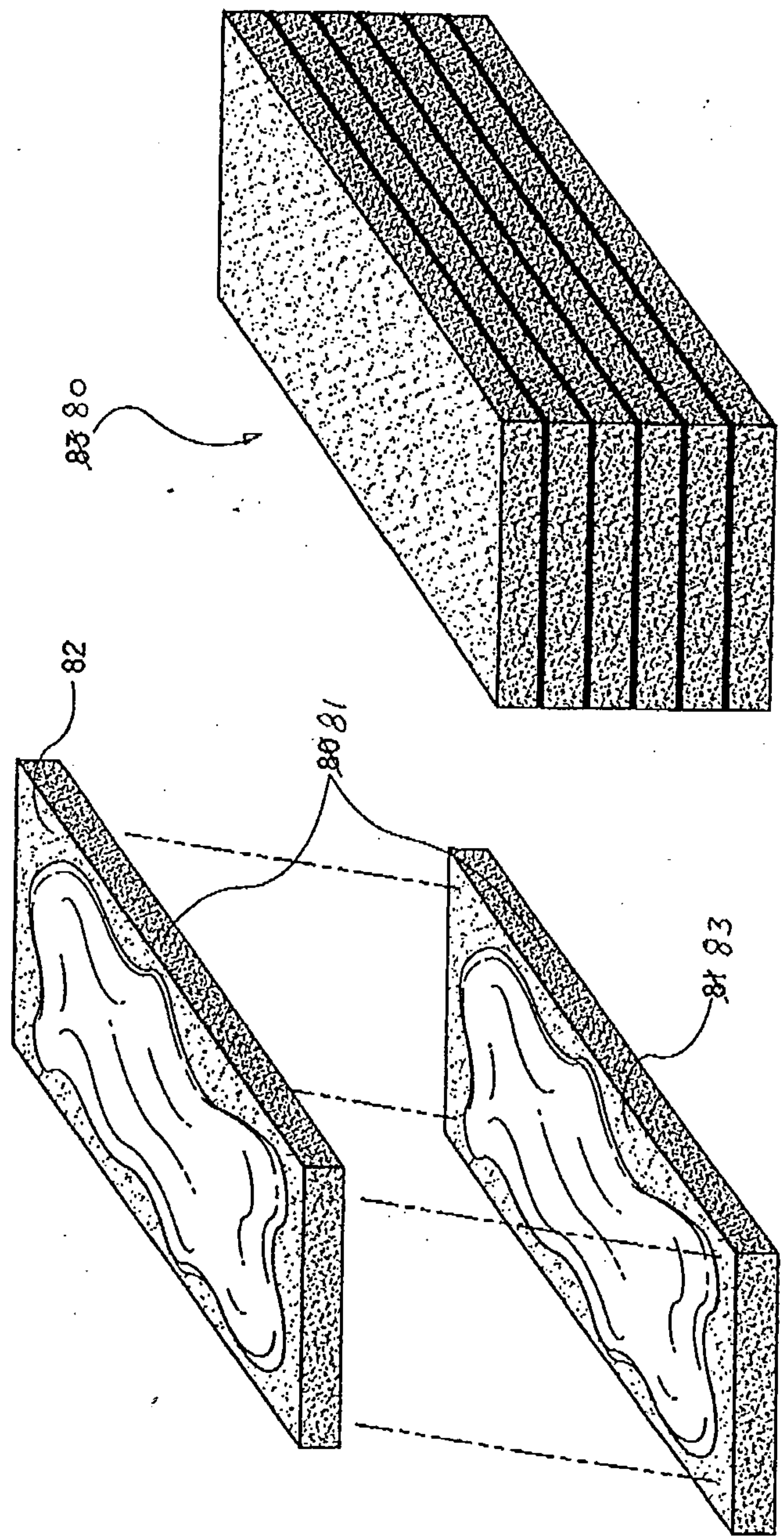

FIG. 8A

FIG. 8

Exhibit B

OSTEOTECH
Innovators in Musculoskeletal Science

FAX

Osteotech, Inc.
51 James Way
Eatontown, NJ 07747

To:	Anthony Bottino/Peter Dilworth
Company:	
Fax number:	+1 (516) 228-8516
Business phone:	
From:	Todd M. Boyce, Ph.D.
Fax number:	+1 (732) 542-0232
Business phone:	(732) 542-2800, ext. 6235
Home phone:	
Date & Time:	1/28/98 11:05:36 AM
Pages:	5
Re:	Comments/Changes on Application 285-79

Following are my comments on the draft patent application.

Todd Boyce

Todd Boyce

OSTEOTECH

Memorandum

DATE: January 28, 1998

TO: Anthony Bottino, Esq./ Dilworth & Barrese

CC: Peter Dilworth, Esq./ Dilworth & Barrese

FROM: Todd Boyce

RE: Application 285-79

My colleagues and I have read the draft of the "cross-linking" patent application 285-79. Below are our suggested changes. If you have any questions, or would like to discuss these comments, you can call me at (732) 544-6235. Thank you.

Page/Line	Change to read	Comment
4/1	—	Is the descriptor "initially presented" a necessary part of this sentence? I find it confusing.
5/16	"... non-antigenic and to provide good mechanical strength."	Was awkward as written in draft.
6/1	"osteoinductive" should be "osteoconductive"	Terms "osteoinductive" and "osteoconductive" were transposed in the first two paragraphs.
6/5	"osteoconductive" should be "osteoinductive."	Terms "osteoinductive" and "osteoconductive" were transposed in the first two paragraphs.
6/12	"... including particles, fibers, strips, ..."	Include "fibers" in the list of shapes covered.
6/21	"The partial demineralization process produces bone-derived elements having an surface binding region, the exposed collagen, and at the same time retaining a strengthening region, the mineralized core of the bone-derived elements."	Add after the end of line 21 to clarify the role of surface demineralization.
8/1	—	Typo: Add a space between "intertransverse" and "process."
9/1	"... and metallic ions, for example ions of iron and aluminum."	Clarifying that ions of iron and aluminum are used.
7/14	"... fashioned as a femoral bone replacement;"	Typo: Change "femural" to "femoral."
9/10-11	"... on the order of from about 10MPa to about 200 MPa, and preferably from about 20 to about 100	Values to complete the blanks.

	MPa."	
9/13	"... original mineral content or, stated and other..."	Typographical error: "of" should be changed to "or."
10/1	"... demineralized bone in any form, including particles, sheets, powder, or shaped demineralized bone pieces, graphite or pyrolytic carbon, bioglass or other...."	We want the application to include the addition of demineralized bone in other forms in addition to powder; we also wanted to add graphite and pyrolytic carbon to the list.
10/5	"... and nonbioabsorbable materials such as starches, polymethyl methacrylate, polytetrafluoroethylene, polyurethane, polyethylene and nylon."	To complete the bracketed list. Changes "polymers" to "materials."
11/12	——	Should "dextroal" be "dextrose?" This seems to be a typo.
11/23	Remove "powder" after "demineralized bone"	We want to include demineralized bone in all of its forms.
12/3	"... immunosuppressants; angiogenic agents, such as basic fibroblast growth factor (bFGF); permeation enhancers..."	Add angiogenic agents to the list.
13/10-11	"... shaped as a bone or section thereof, or as an implant for grafting.	Add the concept of implant (not necessarily shaped as a bone) to the preferred forms.
14/23	——	Typo: "inlay" should be "onlay."
15/20	"... and, enzymatic treatment to form chemical linkages..."	Enzymatic treatment is probably not the "preferred" form.
16/17	——	Typo: "carbiimide" should be "carbodiimide."
17/14	"The osteoimplant is then washed to remove all leachable traces of the chemical.	Change "chemical agent" to "osteoimplant."
17/25	"... accomplished by the application of energy. Energy in the form of ultraviolet light, microwaves and the like may be used to cross-link collagen molecules, most commonly by forming highly reactive oxygen ions generated from the atmospheric gas, which then promote oxygen cross-links between the collagen molecules. With the use of a chemical dye, visible light may also be used to cross-link collagen, via a process known as dye-mediated photo-oxidation."	Completion of the blank in the draft.
18/2	"... chemical linkages is by dehydrothermal treatment. Dehydrothermal treatment uses combined heat and the slow removal of water under a vacuum, to promote crosslinking of the bone-derived elements. The process involves a hydroxy group from a functional group of one molecule and a hydrogen ion from a functional group of another	Completion of the blank in the draft.

	molecule reacting to form water, which is removed, with a resulting new bond between the collagen molecules."	
18/22	"The total thickness of the osteoimplant will ordinarily be at least about 2–20 mm.	Completion of the blank in the draft.
20/24	—	Typo: "0.6 NH_4Cl" should be 0.6N HCl"
21/2	"... assembled into a layered structure and held with a clamp."	Adds the word "and" and changes "in" to "with."
21/7	"... was placed in a container and allowed to rinse under running water for several hours."	Correction of typo.
21/10	"After seven hours, the excess solution was removed, and the osteoimplant was freeze-dried."	Remove the word "glycerol" from the sentence.
21/22	"... poured through a 106 micron sieve to recover the fibers. The mixture of mineralized and demineralized fibers was placed in a cylindrical die, and pressure-treated to 10,000-50,000psi in a press ..."	Addition of detail to the description of example 2. Is it necessary to be specific (i.e. 10,000psi rather than 10,000-50,000psi) in the example?
22/20	"... approximately 1mm thick were surface..."	Typo: Remove "sheets" after the word "thick."
22/21-22	"Tissue transglutaminase was reconstituted..."	Typo: "transglutaminase."
24/1	"What is claimed is:"	Rather than "In the Claims:"
24/Claim 2	"... strips or sheets of allogenic and/or xenogenic cortical or cancellous bone."	Add coverage for cancellous bone treated in the same way.
24/claim 3	"... sheets of allogenic and/or xenogenic cortical or cancellous bone."	Add coverage for cancellous bone treated in the same way.
24/Claim 5	"... bone-growth inducing substance, growth factors, fully mineralized allogenic or xenogenic bone, cellular material, genetic material, calcification-controlling agent, hydration agent inorganic compounds and polymers.	Completion of blanks in the draft.
25/Claim 11	"... possessing a compression strength of from about 10 MPa to about 200 MPa.	Completion of blanks in the draft.
26/Claim 12	"... possessing a compression strength of from about 20 to about 100 MPa.	Completion of blanks in the draft.
27/Claim 20	—	Typo: Comma after "a cranial bone"
27/Claim 21	"... wherein the chemical linkages are formed by the reaction of functional groups of the chemical agent with functional groups of the amino acids in collagen to form bonds within or between collagen molecules."	Completion of the claim.
27/Claim 22	"... crosslinking agent is selected from the group consisting of mono- and dialdehydes, polyepoxy	Completing the blanks for the claim.

?

	compounds, polyvalent metallic oxides, organic tannins, phenolic oxides, sugars, dicyclohexyl carbodiimide, and hexamethylene diisocyanate and similar compounds."	
28/Claim 23	"... chemical linkages are formed by irradiation of the bone-derived elements in a gaseous environment, providing oxygen ions which react to form cross-links with the collagen.	Completion of the blanks for the claim.
28/Claim 24	"... chemical linkages are formed by the removal of water from the bone-derived elements in the presence of heat and/or vacuum.	Completion of the blanks for the claim.
28/Claim 25	"... chemical linkages are formed by enzymatic catalysis of reactive groups between amino acids of collagen molecules.	Completion of the blanks for the claim.
28/Claim 26	"The method of Claim 17 wherein the chemical linkages are formed by irradiation with energy in the presence of a dye."	Additional claim to cover Dye-mediated photo-oxidation. Perhaps it should be a sub-claim of Claim 23 instead?

98_0126a

Exhibit C

OSTEOTECH Inc

Innovators in Musculoskeletal Science

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FEB -6 1998
DILWORTH & BARRESE

February 5, 1998

Anthony Bottino, Esq.
Dilworth & Barrese, Attorneys at Law
333 Earle Ovington Blvd.
Uniondale, NY 11553

Dear Anthony:

Enclosed please find the signed originals for patent application 285-79, and the declaration of small entity status that has been signed by Michael Jeffries, an Osteotech Officer.

Please proceed with filing this application.

Sincerely,

Todd M. Boyce, Ph.D.
Scientist, Research & Development

98_0205a

51 James Way • Eatontown, New Jersey 07724 • (732) 542-2800